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# Oil concentration and fatty acid profile of wild *Helianthus* species from the southeastern United States☆

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ABSTRACT

Sunflower (*Helianthus annuus* L.) oil has the potential to be improved for industrial and nutritional purposes through selection and breeding. The narrow genetic base of cultivated sunflower has been broadened and agronomic traits have been enhanced by the infusion of genes from wild species. Interest in using wild species in breeding programs has increased, but information about oil concentration and fatty acid composition is lacking for a number of rare and threatened species. The objective of this study was to evaluate achenes of seven wild sunflower species from the southeastern USA: *H. eggertii*, *H. schweinitzii*, *H. porteri*, *H. verticillatus*, *H. smithii*, *H. angustifolius*, and *H. atrorubens*, for oil concentration and fatty acid composition of four major fatty acids, palmitic, stearic, oleic, linoleic acids; and five minor acids, myristic, linolenic, arachidic, behenic, and lignoceric. Achenes of all populations were collected throughout the distributional range of the species. *H. verticillatus* had the highest oil concentration of the seven species with 323.4 g/kg, and was within the range reported for other wild perennial sunflower species. The average linoleic acid concentration in *H. porteri* of 817 g/kg is the highest concentration reported for a wild sunflower species. Linoleic acid concentrations for all seven species were higher than normally observed in populations grown in southern latitudes. The saturated palmitic and stearic fatty acids in *H. porteri* total 88 g/kg, about 30% less than cultivated sunflower oil with approximately 120 g/kg. The lower saturated fatty acid profile and the high linoleic concentration in the oil of *H. porteri* indicate that this species has the potential to reduce saturated fatty acids and increase linoleic acid concentration in traditional commercial sunflower oil. Further research will be needed to determine the inheritance of the fatty acids and oil concentration. Other agronomic traits will need to be maintained during the introgression of these traits into sunflower.

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## 1. Introduction

Plant lipid syntheses in vascular plants produce many compounds and secondary metabolites, such as oil. Although oil concentrations of up to 37 g/kg have been reported in whole plants of wild sunflower (*Helianthus annuus* L.), the achenes are the primary storage organ for oil (Seiler et al., 1990). Oil concentration in achenes of the wild desert species, *Helianthus anomalus* Blake from Utah, has been reported to be as high as 460 g/kg (approximately equal to cultivated sunflower) (Seiler, 2007). The oil that accumulates in the achenes of wild and cultivated sunflower is composed of triacylglycerols that exist in the liquid form at room temperature and have a low melting point. The fatty acid (FA) composition of the achene oil determines its end use suitability. Sunflower oil is considered a high quality edible oil; however, the potential for improved industrial and nutritional characteristics through breeding and selection have not been fully evaluated.

Since the development of high-oleic sunflower hybrids, sunflower oil has become a more important feedstock for the oleochemical industry, including the cosmetics industry (Luhs and Friedt, 1994). Sunflower oil is a source of fatty molecules that can be used as reagents for chemical modification (Girardeau et al., 2000; Leyris et al., 2000). These can be used in the manufacture of lacquers, copolymers, polyester films, modified resins, and plasticizers, when there is a price advantage to the manufacturer. Sunflower oil is currently being used in the manufacture of alkyd resins for coatings from recycled polyethylene terephtalate (PET) (Dullius et al., 2006).

Emulsifiers and surfactants from sunflower oil are used in formulating pesticides (Pryde and Rothfus, 1989). Development of the mid-oleic NuSun® oil in the USA has increased the demand for this

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type of oil in the food processing industry due to its high oxidative stability (Kleingartner, 2002) and potential for increasing the oil stability index of biodiesel (Moser, 2008).

Biodiesel, an alternate fuel composed of monoalkyl esters of long-chain fatty acids prepared from vegetable oils or animal fats, has attracted considerable interest as a substitute or blend component for ultra-low sulfur diesel fuel (Moser, 2008). High-oleic sunflower oil has been evaluated as a potential for biodiesel blend (Moser, 2008). The influence of the fatty acid composition of high-oleic sunflower oil biodiesel has been studied by Ramos et al. (2009) and Pereyra-Irujo et al. (2009). It is estimated that if a farmer in North Dakota, USA, devotes 10% of their production acreage to oilseed sunflower production for fuel, the total on-farm fuel requirement could be met (Hofman and Hauck, 1982). Feedstock costs will ultimately determine the extent of the use for NuSun® and high-oleic sunflower oils.

Oil concentration and fatty acid composition, especially oleic and linoleic fatty acids, of oil from wild and cultivated sunflower vary greatly, mainly as a response to temperature during seed development (Harris and James, 1969; Harris et al., 1978; Seiler, 1986). A high temperature during seed maturation results in oil with high oleic acid concentration, and a low linoleic acid concentration. Generally, the cooler northern latitudes produce higher concentrations of linoleic acid in the oil than the warmer southern latitudes (DeHaro and Fernandez-Martinez, 1991).

The genus *Helianthus* consists of 51 species and 19 subspecies with 14 annual and 37 perennial species (Schilling, 2006). The narrow genetic base of cultivated sunflower has been broadened by the infusion of genes from wild species. This has resulted in continuous improvement of agronomic and economic traits in cultivated sunflower (Thompson et al., 1981; Seiler, 1992; Seiler and Rieseberg, 1997; Jan and Seiler, 2007). Recent emphasis on the oil concentration and fatty acid composition of sunflower achenes has increased interest in using wild species in breeding programs to enhance oleic or linoleic acid, or to reduce saturated fatty acids.

While a few populations of some wild sunflowers have been collected and evaluated for oil concentration and fatty acid composition, many remain to be evaluated to fully characterize the available genetic variability. There is an urgent need to collect and evaluate those that are endemic to limited geographic areas and may be at risk of being eliminated by human activities and other changes such as climate change. The objective of this study was to evaluate achenes of seven wild sunflower species: *H. eggertii* Small, *H. schweinitzii* T. & G., *H. porteri* (A. Gray) Heiser, *H. verticillatus* E. E. Watson, *H. smithii* Heiser, *H. angustifolius* L., and *H. atrorubens* L. collected from the southeastern region of the USA for oil concentration and fatty acid composition of four major fatty acids, palmitic, stearic, oleic and linoleic acids; and five minor acids, myristic, linolenic, arachidic, behenic, and lignoceric.

## 2. Materials and methods

### 2.1. Plant materials

Populations of wild sunflowers were collected between 17 and 28 October 2003. The exploration covered a distance of 4600 km in five USA states: Alabama, Georgia, North Carolina, South Carolina, and Tennessee (Gulya et al., 2007). Heads of wild sunflowers were collected from 50 to 250 plants within each population and bulked into a single sample. Herbarium specimens were deposited at the USDA-ARS wild *Helianthus* herbarium at Fargo, North Dakota. Achene samples were sent to the USDA-ARS North Central Regional Plant Introduction Station, Ames, Iowa, USA where they are maintained and distributed. Each population of a species was assigned a Plant Introduction (PI) number or an A number (Ames numbers are used prior to assigning a PI number) or a species identification (SCH = *schweinitzii*) plus a population number for identification purposes.

All populations were collected throughout the broad distributional range of the species. Prior collection sites obtained from herbarium specimens and generalized distribution maps were used to locate populations. Population size (number and extent), habitat, soil type, achene set per head, the presence of diseases and insects, GPS coordinates including elevation, and the presence of other wild sunflower species located near the collection sites were recorded for each population (Gulya et al., 2007).

### 2.2. Oil and fatty acid analyses

Achenes were stored at 5 °C and low humidity (<20%) until analyzed. Each sample represented an isolated, open-pollinated segregating population. Two 6-ml achene samples from each population were cleaned to remove empty achenes, and two subsamples of each sample were analyzed for oil concentration (expressed as a percent on a dry weight basis) by nuclear magnetic resonance (Granlund and Zimmerman, 1975). Fatty acid composition was determined from a 10 achene composite sample from each sample and subsample used for oil analysis. A small portion of the pulverized 10 achene sample (10–20 mg) was transferred to a disposable filter column (Fisher Scientific, Pittsburgh, PA) and eluted with 1.5 ml of a hexane–chloroform–0.5 mol $dm^{-3}$ sodium methoxide in methanol (75:20:5, v/v) solution added to a glass vial and capped (Vick et al., 2004). The sample was injected into a Hewlett-Packard 5890 gas chromatograph containing a DB-23 capillary column (30 m × 0.25 mm, J & W Scientific, Folsom, CA), which was held at 190 °C for 5 min, then increased to 220 °C at 10 °C/min, held at 220 °C for 1 min, and then increased to 240 °C at 20 °C/min, and finally held at 240 °C for 1.5 min, for a total time of 11.5 min. The detector was a flame ionization detector (FID). A fatty acid standard, 21A (Nu-Chek-Prep, Inc, Elysian, MN), containing methyl esters of the following acids: palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and arachidic (20:0), 11-eicosenoic (20:1), behenic (22:0), and lignoceric (24:0) was used as a reference. Fatty acid peaks were identified by comparing the fatty acid methyl ester peaks and retention time of the standard with the sample peaks. Chemstation software was used to calculate the area under each peak, and fatty acid concentrations were expressed as a percentage by weight of the total fatty acids. Fatty acid and oil concentrations were means of two samples subsampled twice per population.

### 2.3. Data analysis

The data were analyzed using an analysis of variance ANOVA (SAS®, 2009). Means were separated using Duncan's new multiple range test. Standard error was calculated across all populations and species. Since the species populations were not grown in a common location, the data was confounded by environmental differences, and the analysis was treated conservatively by nesting the populations within species and analyzing the data as a mixed model where the location was a variable (random sample) and the species were fixed effects.

## 3. Results

### 3.1. Collection of populations

Mature achenes were collected from five rare species: four perennial, *H. eggertii*, *H. schweinitzii*, *H. verticillatus*, and *H. smithii*; and one annual, *H. porteri* (Table 1). Two additional perennial species with wider distributional ranges, *H. angustifolius* and *H. atrorubens* were also collected. *H. porteri* is a rare annual species that

**Table 1**
Wild *Helianthus* populations collected for oil concentration and fatty acid composition of seven species from the southeastern USA.

| Species | Number of populations | State (location) | Habitat |
|---|---|---|---|
| Annual | | | |
| *H. porteri* | 8 | Georgia and North Carolina | Granite outcrops, Piedmont Region |
| Perennial | | | |
| *H. angustifolius* | 2 | Georgia and Tennessee | Open to shaded woods, usually moist places |
| *H. atrorubens* | 1 | Alabama | Open mixed woods, dry roadsides, dry shaded hillsides |
| *H. eggertii* | 13 | Alabama, South Carolina, and Tennessee | Grassy openings, barrens, open oak-hickory woods |
| *H. schweinitzii* | 14 | North and South Carolina | Open woodlands, clearings, Piedmont region |
| *H. smithii* | 1 | North Carolina | Dry, open woods |
| *H. verticillatus* | 2 | Alabama and Tennessee | Moist, prairie-like openings, and edge of woodlands, clay soil |

was recently transferred from the genus *Viguiera* (Pruski, 1998). Eight populations of this species were collected from its known distributional range in Georgia and North Carolina. Two populations of diploid perennial *H. angustifolius* were collected in Georgia and Tennessee, and one population of *H. atrorubens* was collected in Alabama. *H. eggertii* is a hexaploid perennial species that was recently removed from the threatened species list by the U.S. Fish and Wildlife Service (USFWS, 2005). Thirteen populations were collected from most of all areas for the species range, Alabama, South Carolina, and Tennessee, but not Kentucky. *H. schweinitzii* is a federally protected rare hexaploid species in the Piedmont region of North and South Carolina. Fourteen populations of this species were collected from throughout its range. *H. smithii*, a rare diploid perennial species, was collected from a single population in North Carolina, near the eastern edge of its distributional range. Perennial *H. verticillatus* is a species which was described over 100 years ago (Small, 1898), but was not rediscovered or recollected until recently in Tennessee (Mathews et al., 2002). Two populations of this species were collected: one from Tennessee and one from Alabama.

### 3.2. Variation in oil concentration among species

Differences in oil concentration among species were not statistically significant. Oil concentration in achenes varied among the species, averaging 295 g/kg (Table 2 ). Oil concentrations in achenes of *H. porteri* populations varied from 318 g/kg in PI 649914 to 269.5 g/kg in PI 649172, averaging 291 g/kg. Oil concentration of two diploid perennial *H. angustifolius* populations and one diploid perennial *H. atrorubens* population averaged 298.2 and 280 g/kg, respectively. *H. eggertii* populations averaged 288.7 g/kg, ranging from 331.5 in A 27676 to 245.5 g/kg in A 27681, respectively. The oil concentration for the 14 populations of *H. schweinitzii* averaged 286.3 g/kg and ranged from 320 in SCH-2409 to 234.5 g/kg in SCH-2405. The oil concentration for the single population of *H. smithii* (PI 650085) was 298.5 g/kg. Oil concentrations in the newly rediscovered species *H. verticillatus* averaged 323.4 g/kg, ranging from a high of 346.0 in PI 650119 to 301.5 g/kg in PI 650110. This was the highest oil concentration of any species population collected and the first report of oil concentration for this species.

### 3.3. Variation in fatty acids among species

The average concentration of the four major fatty acids (FA), palmitic, stearic, oleic, and linoleic acids in oil varied among the *Helianthus* species (Table 2). In this study, the most abundant FA was linoleic acid, found in all species averaging 763.8 g/kg, while oleic acid averaged 116.3 g/kg and palmitic and stearic averaged 56.4 and 33.6 g/kg, respectively. The 817.1 g/kg average linoleic concentration in *H. porteri* was the highest observed in any wild sunflower species, with PI 649911 having a concentration of 834.4 g/kg, the highest ever observed for a wild sunflower population. Among the seven species, the lowest average oleic acid concentration, 65.4 g/kg, was observed in *H. porteri*, with the lowest observed in PI 649916 (45.1 g/kg). The average concentration of oleic FA for all species was 116.3 g/kg, and was almost twice that of the average for *H. porteri*. The concentration of saturated palmitic and stearic FA in *H. porteri* averaged 55.6 and 32.2 g/kg, respectively, compared to the average of all species reaching 56.4 and 33.6 g/kg, respectively.

Oleic FA concentration of *H. angustifolius* (PI 649936 and PI 649937) averaged 118.7 g/kg and *H. atrorubens* (PI 649940) averaged 93.1 g/kg. Average linoleic FA concentration for *H. angustifolius* was 720.1 g/kg and 783.4 g/kg for *H. atrorubens*. Palmitic and stearic acids averaged 75.0 and 38.4 g/kg for *H. angustifolius*, and 64.6 and 35.0 g/kg for *H. atrorubens*, respectively.

In the 13 *H. eggertii* populations, oleic FA averaged 155.0 g/kg, ranging from a high of 185.3 g/kg in A 27680 to a low of 125.1 g/kg in PI 649976. The linoleic FA averaged 728.2 g/kg, ranging from a high of 772.5 g/kg in PI 649976 to a low in PI 649981 of 582.2 g/kg. The saturated palmitic and stearic FA averaged 55.6 and 28.8 g/kg, respectively in *H. eggertii*. The accession, PI 649981 produced the highest palmitic FA reaching 107.1 g/kg and A 27681 had the highest stearic FA reaching 35.3 g/kg.

Among the 14 *H. schweinitzii* populations, oleic and linoleic FA averaged 107.5 and 765.2 g/kg, respectively, ranging from a high of 129.9 for oleic FA in SCH-2403 to a low of 94.0 g/kg in SCH-2404, and from a high of 805.4 in SCH-2411 to a low of 755.8 g/kg in SCH-2414 for linoleic FA. Saturated palmitic and stearic FA averaged 54.8 and 39.6 g/kg, respectively with a high of 58.9 g/kg of palmitic FA in SCH-2415 and 48.7 g/kg of stearic FA in SCH-2414.

The only population of *Helianthus smithii* (PI 650085) collected had an oleic and linoleic FA concentration of 106.7 and 788.4 g/kg, respectively. Palmitic and stearic FA concentrations were 51.7 and 33.2 g/kg.

The recently rediscovered perennial *H. verticillatus* had an oleic FA concentration that averaged 138.3 g/kg and a linoleic concentration of 749.2 g/kg. The saturated palmitic and stearic FA averaged 56.6 and 25.8 g/kg, respectively. This is the first report for the fatty acid composition in the oil of this species.

The other five FA in sunflower oil, myristic, linolenic, arachidic, behenic and lignoceric were present in minor amounts in all populations, usually accounting for less than 2% of the oil composition. Statistical comparison of species means for the minor FA showed a significant difference for all FA, except myristic acid. The myristic FA concentration averaged 0.47 g/kg (Table 2). Linolenic acid averaged 2.39 g/kg across all species, with *H. atrorubens* (PI 649940) having the highest concentration, 4.13 g/kg. Of the 41 populations, the highest concentration of arachidic acid was observed in *H. schweinitzii* (SCH-2410) with 5.14 g/kg compared to the grand mean of 3.19 g/kg. Over all species, behenic and lignoceric FA averaged 5.45 and 1.34 g/kg, with the highest concentrations of behenic FA observed in *H. schweinitzii* (SCH-2410) with 8.32 and 2.04 g/kg of lignoceric observed in *H. schweinitzii* (SCH-2405).

**Table 2**
Oil concentration and fatty acid composition of seven *Helianthus* species collected from the southeastern USA.

| Species | Oil concentration (g/kg) | Myristic (g/kg) | Palmitic (g/kg) | Stearic (g/kg) | Oleic (g/kg) | Linoleic (g/kg) | Linolenic (g/kg) | Arachidic (g/kg) | Behenic (g/kg) | Lignoceric (g/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| Annual | | | | | | | | | | |
| *porteri* | | | | | | | | | | |
| PI 649911[a] | 317.0 | 0.40 | 47.6 | 23.5 | 68.1 | 834.4 | 1.79 | 1.72 | 2.06 | 0.64 |
| PI 649912 | 281.5 | 0.47 | 57.5 | 34.4 | 78.5 | 797.7 | 2.31 | 2.61 | 3.31 | 1.02 |
| PI 649913 | 301.5 | 0.45 | 55.3 | 35.4 | 69.4 | 814.1 | 2.04 | 2.40 | 2.51 | 0.76 |
| PI 649914 | 318.0 | 0.44 | 56.4 | 29.5 | 61.1 | 825.3 | 1.93 | 2.17 | 2.19 | 0.73 |
| PI 64915 | 289.9 | 0.44 | 57.0 | 36.6 | 57.4 | 820.4 | 2.47 | 2.19 | 4.38 | 0.79 |
| PI 649916 | 280.5 | 0.44 | 61.0 | 36.4 | 45.1 | 828.1 | 2.39 | 2.69 | 3.60 | 1.00 |
| PI 649172 | 269.5 | 0.48 | 56.8 | 30.7 | 78.4 | 799.8 | 2.55 | 2.12 | 3.36 | 0.81 |
| PI 649918 | 271.5 | 0.44 | 52.7 | 31.5 | 65.3 | 817.5 | 2.35 | 2.25 | 4.16 | 0.89 |
| Mean ± SE[b,c] | 291.0 ± 4.7 ns | 0.44 ± 0.00 ns | 55.6 ± 0.95 b | 32.2 ± 1.1 bc | 65.4 ± 2.7 d | 817.1 ± 3.2 a | 2.23 ± 0.07 b | 2.27 ± 0.07 d | 3.20 ± 0.21 c | 0.83 ± 0.03 d |
| Perennial | | | | | | | | | | |
| *angustifolius* | | | | | | | | | | |
| PI 649936 | 280.5 | 0.44 | 74.4 | 38.5 | 132.9 | 683.3 | 2.78 | 3.45 | 6.30 | 1.71 |
| PI 649937 | 316.0 | 0.44 | 75.6 | 38.4 | 104.5 | 757.8 | 2.77 | 3.15 | 5.49 | 1.84 |
| Mean ± SE | 298.2 ± 10.3 ns | 0.42 ± 0.02 ns | 75.0 ± 0.07 a | 38.4 ± 0.29 ab | 118.7 ± 8.4 b | 720.1 ± 21.9 d | 2.78.2 ± 0.03 b | 3.30 ± 0.09 bc | 5.90 ± 0.23 ab | 1.76 ± 0.03 a |
| *atrorubens* | | | | | | | | | | |
| PI 649940 | 280.0 ± 1.2 ns | 0.54 ± 0.02 ns | 64.6 ± 1.24 ab | 35.0 ± 0.994 ac | 93.1 ± 3.49 c | 783.4 ± 4.99 ab | 4.13 ± 0.05 a | 2.74 ± 0.07 cd | 6.48 ± 0.05 a | 1.05 ± 0.03 cd |
| *eggertii* | | | | | | | | | | |
| PI 649974 | 272.0 | 0.44 | 49.1 | 24.3 | 158.6 | 727.6 | 1.33 | 2.23 | 4.36 | 1.14 |
| A 27676[d] | 331.5 | 0.34 | 47.7 | 28.7 | 163.7 | 733.2 | 1.01 | 2.51 | 4.51 | 1.27 |
| PI 649975 | 296.5 | 0.36 | 54.4 | 25.7 | 166.3 | 730.0 | 3.48 | 2.22 | 4.61 | 1.26 |
| PI 649976 | 306.0 | 0.40 | 47.9 | 33.1 | 125.1 | 772.5 | 1.22 | 2.70 | 4.93 | 1.54 |
| PI 649977 | 309.0 | 0.39 | 48.1 | 31.9 | 140.0 | 759.6 | 1.06 | 3.17 | 5.87 | 1.48 |
| A 27680 | 300.5 | 0.56 | 52.7 | 27.6 | 185.3 | 712.8 | 0.88 | 2.35 | 6.13 | 1.56 |
| A 27681 | 245.5 | 0.44 | 53.0 | 35.3 | 175.8 | 719.0 | 1.29 | 3.56 | 6.68 | 1.43 |
| PI 649928 | 294.0 | 0.44 | 44.1 | 32.1 | 179.1 | 717.7 | 0.80 | 2.83 | 6.35 | 1.57 |
| PI 649979 | 256.5 | 0.45 | 67.4 | 24.8 | 153.1 | 730.9 | 1.33 | 2.30 | 6.46 | 1.49 |
| PI 649980 | 255.0 | 0.39 | 47.6 | 27.3 | 145.8 | 764.6 | 1.11 | 2.09 | 4.94 | 1.08 |
| PI 649981 | 247.5 | 0.94 | 107.1 | 32.3 | 144.2 | 582.2 | 3.22 | 3.21 | 5.12 | 1.13 |
| PI 649982 | 323.5 | 0.75 | 47.0 | 25.9 | 133.2 | 767.3 | 1.71 | 2.24 | 4.95 | 1.34 |
| PI 649983 | 316.5 | 0.85 | 55.8 | 25.9 | 145.5 | 750.3 | 1.81 | 2.64 | 7.73 | 0.97 |
| Mean ± SE | 288.7 ± 5.82 ns | 0.53 ± 0.04 ns | 55.6 ± 3.19 b | 28.8 ± 0.70 cd | 155.0 ± 3.60 a | 728.2 ± 9.43 cd | 1.6 ± 0.02 b | 2.62 ± 0.09 d | 5.59 ± 0.20 ab | 1.33 ± 0.04 bc |
| *schweinitzii* | | | | | | | | | | |
| SCH-2403[e] | 293.0 | 0.34 | 47.8 | 35.8 | 129.9 | 765.4 | 1.62 | 4.11 | 7.02 | 2.02 |
| SCH-2404 | 275.5 | 0.58 | 57.7 | 42.5 | 94.0 | 779.6 | 3.41 | 4.21 | 6.22 | 1.25 |
| SCH-2405 | 234.5 | 0.56 | 58.4 | 34.1 | 94.9 | 781.9 | 2.93 | 4.62 | 6.16 | 2.04 |
| SCH-2406 | 310.5 | 0.49 | 54.2 | 46.8 | 118.9 | 756.4 | 2.77 | 4.85 | 6.39 | 1.77 |
| SCH-2407 | 316.5 | 0.39 | 55.8 | 40.1 | 111.6 | 767.8 | 2.84 | 4.83 | 7.20 | 1.70 |
| SCH-2408 | 260.0 | 0.45 | 57.0 | 48.5 | 106.5 | 762.2 | 2.74 | 4.79 | 6.72 | 1.71 |
| SCH-2409 | 320.0 | 0.43 | 47.2 | 38.7 | 100.5 | 786.9 | 2.13 | 3.87 | 7.37 | 1.83 |
| SCH-2410 | 249.0 | 0.43 | 57.2 | 42.3 | 105.6 | 769.3 | 2.47 | 5.14 | 8.32 | 1.56 |
| SCH-2411 | 316.5 | 0.30 | 51.1 | 29.2 | 95.1 | 805.4 | 2.01 | 2.70 | 4.48 | 1.24 |
| SCH-2412 | 307.0 | 0.44 | 53.9 | 36.0 | 104.8 | 772.7 | 2.17 | 3.93 | 6.02 | 1.06 |
| SCH-2414 | 254.5 | 0.45 | 57.7 | 48.7 | 104.9 | 755.8 | 2.68 | 4.77 | 7.22 | 1.52 |
| SCH-2415 | 285.5 | 0.41 | 58.9 | 33.7 | 115.4 | 756.6 | 2.59 | 4.15 | 5.84 | 1.56 |
| SCH-2416 | 299.5 | 0.45 | 54.9 | 38.4 | 115.4 | 766.5 | 2.45 | 4.55 | 6.67 | 1.44 |
| Mean ± SE | 286.3 ± 5.61 ns | 0.44 ± 0.02 ns | 54.8 ± 0.77 b | 39.6 ± 1.16 a | 107.5 ± 2.12 bc | 765.2 ± 2.08 ac | 2.53 ± 0.09 b | 4.35 ± 004 a | 6.59 ± 0.18 a | 1.59 ± 0.57 ab |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *smithii* | | | | | | | | | | |
| PI 650085 | 298.5 ± 2.49 ns | 0.46 ± 0.02 ns | 51.7 ± 0.77 b | 33.2 ± 0.05 ac | 106.7 ± 2.49 bc | 788.4 ± 10.2 ab | 1.73 ± 0.05 b | 2.43 ± 0.04 d | 5.73 ± 0.05 ab | 1.31 ± 0.01 bc |
| *verticillatus* | | | | | | | | | | |
| PI 650119 | 346.0 | 0.44 | 55.1 | 24.9 | 153.6 | 733.0 | 1.69 | 3.57 | 5.45 | 1.77 |
| PI 650110 | 301.5 | 0.53 | 58.1 | 26.7 | 123.0 | 766.5 | 2.38 | 3.62 | 4.58 | 1.47 |
| Mean ± SE | 323.4 ± 12.9 ns | 0.49 ± 0.03 ns | 56.6 ± 1.03 b | 25.8 ± 0.6 d | 138.3 ± 9.0 a | 749.3 ± 9.8 bd | 2.04 ± 0.20 b | 3.59 ± 0.25 b | 5.01 ± 0.50 b | 1.61 ± 0.17 ab |
| Overall means ± SE | 295.0 ± 18.7 ns | 0.47 ± 0.01 ns | 56.4 ± 1.2 | 33.6 ± 0.7 | 116.3 ± 4.0 | 763.8 ± 5.1 | 2.39 ± 0.16 | 3.19 ± 0.11 | 5.45 ± 0.17 | 1.34 ± 0.04 |
| Statistical significance | NS | NS | * | ** | ** | ** | ** | ** | ** | ** |

[a] Plant Introduction.
[b] Standard error.
[c] Species means followed by the same letter in a column are not significantly different at the $^{*}P < 0.05$ or $^{**}P < 0.01$ level according to the Duncan's new multiple range test.
[d] Ames number used before a PI number is assigned.
[e] SCH = population collection number.

## 4. Discussion

Wild *Helianthus* populations generally have oil concentrations of 250–350 g/kg (Seiler, 1985, 1994) which is much lower than cultivated sunflower with 440–480 g/kg (Robertson et al., 1979). Oil concentration in achenes of a wild desert species, *H. anomalous* from Utah, has been reported to reach as high as 460 g/kg (approximately equal to cultivated sunflower) (Seiler, 2007). The oil concentrations in the current study are all within the range previously reported for wild sunflower species. Oil concentrations of interspecific hybrids can be rapidly increased to an acceptable level by backcrossing with cultivated sunflower lines (Seiler and Rieseberg, 1997). Based on this fact, there should be little concern about the lower oil concentration of the wild species when they are used as sources of unique traits.

Previously, oil concentration for a single population of *H. porteri* from Georgia was reported to be 174 g/kg (Seiler, 1985). The low concentration may be due to poor achene quality or having examined only a single population not representative of the species. The current study examined eight populations from most of the species' range for oil concentration averaging 291 g/kg and is probably more representative of this species than the previous report.

The average oil concentration of *H. eggertii* in the current study was 288.7 g/kg, and was higher than the 222 g/kg concentration reported by Thompson et al. (1981). Achenes from the original populations were evaluated in both studies. The higher oil concentrations observed in the current study may be more representative of the potential genetic diversity of the species than the concentration reported by Thompson et al. (1981) because of the larger number of populations analyzed from a wider geographic range for *H. eggertii*.

A previous report of oil concentration in *H. schweinitzii* was only 82 g/kg, which is an extremely low value resulting from a very low achene weight indicating incomplete achene filling (Seiler, 1985). The oil concentration of 286.3 g/kg in the current study is closer to that normally observed for a perennial sunflower species from this region (Seiler, 1985). The reported oil concentration for *H. smithii* of 298 g/kg from a single population in the current study is slightly above the range of 247–285 g/kg previously reported for this species (Seiler, 1985).

*H. verticillatus* had the highest average oil concentration of any species in the current study. This is the first report of oil concentration and fatty acid composition of this recently rediscovered species. This species is restricted to three sites, two of which were collected for this study.

The mean oil concentration in *H. angustifolius* in the current study was 298.2 g/kg, slightly higher than the 252 g/kg reported for the single population evaluated by Thompson et al. (1981). The few populations evaluated probably do not represent the total genetic variability since only a small portion of its distributional range was sampled. The oil concentration of *H. atrorubens* averaged 280 g/kg, which is within the range of 211–320 g/kg based on 15 populations reported by Seiler (1985).

It has been established that quantitative variation in fatty acid composition in achene oil can be related to habitat, environment, and genetic differences (Levin, 1974; Linder, 2000; O'Neill et al., 2003). Thompson et al. (1981) concluded that the environments (geographic location) in which collections of different species were made did not influence the oil concentration. The effect of temperature on oil concentration in sunflower is inconsistent. Seiler (1983) showed that environmental factors related to temperature are not related to oil concentration in wild *H. annuus*. Robertson et al. (1979) found that average temperature during full-bloom to harvest stages of field-grown cultivated sunflower in North America had no apparent effect on oil content of seed obtained from 22 locations in 1976 or 35 locations in 1977. Harris et al. (1978)

concluded that oil concentration of cultivated sunflower decreased as temperature increased; whereas Unger and Thompson (1982) observed a decrease in oil content as temperature decreased.

The composition of FA in oil of wild sunflower species is comparable to that observed in cultivated sunflower for the four major and five minor FA (Seiler, 1986). Also, the relationship of environmental factors and FA composition and oil concentration are similar in wild and cultivated sunflower.

The average 817.1 g/kg linoleic FA concentration for *H. porteri* is the highest of any sunflower species. Previous reports for linoleic acid for this species were 834 g/kg in one population from Georgia (Seiler, 1985) and one population regenerated in France with 818 g/kg (DeHaro and Fernandez-Martinez, 1991).

The fact that populations of all seven species had linoleic concentrations >720 g/kg indicates that this trait should have a genetic basis because it is relatively stable in the different populations and species over a wide range of environments (DeHaro and Fernandez-Martinez, 1991). Linoleic sunflower oil with >700 g/kg is preferred for the production of soft margarine (DeHaro and Fernandez-Martinez, 1991).

The linoleic fatty acid concentration observed in the *H. porteri* populations and other rare species is unusually high for being grown in warm southern latitudes. Generally, the cooler northern latitudes have higher concentrations of linoleic FA in the oil because cooler temperatures during flowering, achene filling, and maturation favor a high linoleic concentration and lower oleic FA concentration (DeHaro and Fernandez-Martinez, 1991). A lower concentration of 540 g/kg of linoleic acid is more typical of the concentration expected in warmer southern latitudes (Seiler, 1986, 2007).

Since the higher linoleic FA has been observed in several different species, this may be an adaptation of these species to southern latitudes. The introgression of wild species into cultivated sunflower could facilitate expansion of commercial production of high-linoleic sunflower into the southern latitudes.

There is a strong negative relationship between linoleic and oleic acid concentrations; i.e., if linoleic increases, oleic decreases (Seiler, 1983, 1986). This relationship is common to both wild and cultivated sunflower. Thus, the high average linoleic FA concentration of 817 g/kg in *H. porteri* is accompanied by a corresponding low concentration of oleic acid of approximately 65 g/kg. Low oleic FA concentrations for *H. porteri* from Georgia were reported to be 55 g/kg by Seiler (1985) and, 60 g/kg by DeHaro and Fernandez-Martinez (1991) for one population grown in France. Reports of oleic concentrations from other populations of *H. eggertii* were 210 g/kg compared to 155 g/kg in the current study (Thompson et al., 1981). For *H. smithii*, oleic FA concentration was 190 g/kg for a single population grown in Spain, and 180 g/kg when grown in France (DeHaro and Fernandez-Martinez, 1991). In the present study, oleic FA was 106.7 g/kg and was similar to the 119 g/kg from a single population in North Carolina (Seiler, 1985).

The environmental relations between saturated palmitic and stearic fatty acids are less clear than those for linoleic and oleic FA. In a study based on a few wild sunflower species, those collected from northern latitudes had lower saturated fatty acids than those from further south (Seiler, 1994, 1999). The mean palmitic acid concentration ranged from 51.7 g/kg for *H. smithii* to 75 g/kg in *H. angustifolius*, while stearic acid ranged from 25.8 g/kg in *H. verticillatus* to 39.6 g/kg in *H. schweinitzii*. This is similar to wild *H. annuus* producing 51 g/kg palmitic and 31 g/kg stearic acid (Seiler, 1983). The combined palmitic and stearic FA concentration ranged from 82.4 g/kg in *H. verticillatus* to a high of 113.4 g/kg in *H. angustifolius*. The saturated palmitic and stearic FA totaling 82.4–87.8 g/kg in *H. verticillatus*, *H. smithii*, *H. eggertii*, and *H. porteri* are about 30% less than the linoleic FA concentration of traditional cultivated sunflower oil with approximately 120 g/kg (Dorrell and Vick, 1997). These species offer the potential to introgress reduced saturated FA traits into cultivated sunflower. High concentrations of saturated FA are indicative for high cloud point of methyl esters when used as biodiesel (Muniyappa et al., 1996).

Since oil concentration and oil quality are major traits in sunflower breeding and production, knowledge of these traits in the wild species will help facilitate their use for improvement of cultivated sunflower when these species are used in a breeding program. Lower saturated fatty acids in sunflower oil may be possible by using these species.

Analysis of the five minor FA did not reveal any unusual concentrations of long chain FA (C20–C24). The low concentration of linolenic FA is one desirable factor in sunflower oil. The high concentration of linoleic FA and very low linolenic FA means that despite the moderate iodine number of 125–140 (Salunkhe et al., 1992), it has good drying qualities for lacquers and varnishes without the yellowing associated with high linolenic acid oils (Dorrell and Vick, 1997).

## 5. Conclusions

The introgression of wild species into cultivated sunflower with different fatty acid profiles and a stable linoleic concentration could facilitate the expansion of commercial sunflower production into the southern latitudes. There appears to be sufficient variability to introduce and select for high linoleic acid concentration and reduced saturated fatty acid concentrations in cultivated sunflower oil. Research is needed to determine the inheritance of fatty acid composition and oil concentration. Other agronomic traits will need to be maintained during the introgression of these traits into cultivated sunflower. The addition of these wild species populations to the wild sunflower germplasm collection significantly increases the available genetic diversity for improving cultivated sunflower and also insures their preservation.

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